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PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. ______

Project No. E-19-663 (R6110-0A0) GTRC/GIT DATE 04 / 10 / 86

Project Director: R. S. Roberts & J. D. Muzzy School/Lab ChE

Sponsor: National Science Foundation

Type Agreement: Grant No. CBT-8505960

Award Period: From 3/15/86 To 8/31/87 (Performance) 11/30/87 (Reports)

Sponsor Amount:

	This Change	Total to Date
Estimated:	$	$ 48,211
Funded:	$	$ 48,211

Cost Sharing Amount: $ 3,600 Cost Sharing No: E-19-338

Title: Industry/University Cooperative Research Project: Buffered Solvent Delingnification of Biomass

ADMINISTRATIVE DATA

OCA Contact John B. Schonk X-4820

1) Sponsor Technical Contact:

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Defense Priority Rating: N/A

Military Security Classification: N/A
(or) Company/Industrial Proprietary: N/A

RESTRICTIONS

See Attached NSF Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

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NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 08/16/91

Project No. E-19-663 Center No. R6110-0A0

Project Director ROBERTS R S School/Lab CHEM ENGR

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Contract/Grant No. CBT-8505960 Contract Entity GTRC

Prime Contract No.

Title INDUS/UNIV COOP RES PROJECT: BUFFERED SOLVENT DELINGNIFICATION OF BIOMAS

Effective Completion Date 870831 (Performance) 871130 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

CommentsNSF LINE OF CREDIT AND 98A SATISFY REQUIREMENT FOR FINAL INVOICE AND PATENT REPORT.

Subproject Under Main Project No.

Continues Project No.

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E-19-663

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PI/PD Name and Address

Professor Ronnie S. Roberts
School of Chemical Engineering
Georgia Institute of Technology
Atlanta, GA 30332-0100

NATIONAL SCIENCE FOUNDATION FINAL PROJECT REPORT

PART I - PROJECT IDENTIFICATION INFORMATION

1. Program Official/Org.	James T. Hsu/Division of Chemical, Biochemical, and Thermal Engineering
2. Program Name	Biochemical and Biomass Engineering
3. Award Dates (MM/YY)	From: 3/15/86 To: 11/30/87
4. Institution and Address	Georgia Institute of Technology Atlanta, GA 30332
5. Award Number	CBT-8505960
6. Project Title	Buffered Solvent Delignification of Biomass

This Packet Contains
NSF Form 98A
And 1 Return Envelope

NSF Grant Conditions (Article 17, GC-1, and Article 9, FDP-II) require submission of a Final Project Report (NSF Form 98A) to the NSF program officer no later than 90 days after the expiration of the award. Final Project Reports for expired awards must be received before new awards can be made (NSF Grants Policy Manual Section 677).

Below, or on a separate page, provide a summary of the completed projects and technical information and attach it to this form. Be sure to include your name and award number on each separate page. See below for more instructions.

PART II - SUMMARY OF COMPLETED PROJECT (for public use)

The summary (about 200 words) must be self-contained and intelligible to a scientifically literate reader. Without restating the project title, it should begin with a topic sentence starting the project's major thesis. The summary should include, if pertinent to the project being described, the following items:

- **The primary objectives and scope of the project**
- **The techniques or approaches used only to the degree necessary for comprehension**
- **The findings and implications stated as concisely and informatively as possible**

See Attached

PART III - TECHNICAL INFORMATION (for program management use)

List references to publications resulting from this award and briefly describe primary data, samples, physical collections, inventions, software, etc. created or gathered in the course of the research and, if appropriate, how they are being made available to the research community.

See Attached

Principal Investigator/Project Director Signature	Date

IMPORTANT:
MAILING INSTRUCTIONS
Return this *entire* packet plus all attachments in the envelope attached to the back of this form. Please copy the information from Part I, Block I to the *Attention line* on the envelope.

PART IV — FINAL PROJECT REPORT — SUMMARY DATA ON PROJECT PERSONNEL

(To be submitted to cognizant Program Officer upon completion of project)

The data requested below are important for the development of a statistical profile on the personnel supported by Federal grants. The information on this part is solicited in response to Public Law 99-383 and 42 USC 1885C. All information provided will be treated as confidential and will be safeguarded in accordance with the provisions of the Privacy Act of 1974. You should submit a single copy of this part with each final project report. However, submission of the requested information is not mandatory and is not a precondition of future award(s). Check the "Decline to Provide Information" box below if you do not wish to provide the information.

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	Senior Staff		Post-Doctorals		Graduate Students		Under-Graduates		Other Participants[1]	
	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.
A. Total, U.S. Citizens	2	--	--	--	2	--	1	--	--	--
B. Total, Permanent Residents										
U.S. Citizens or Permanent Residents[2]: American Indian or Alaskan Native . . .										
Asian										
Black, Not of Hispanic Origin										
Hispanic										
Pacific Islander										
White, Not of Hispanic Origin	2	--	--	--	2	--	1	--	--	--
C. Total, Other Non-U.S. Citizens										
Specify Country 1.										
2.										
3.										
D. Total, All participants (A + B + C)	2	--	--	--	2	--	1	--	--	--
Disabled[3]										

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[1]Category includes, for example, college and precollege teachers, conference and workshop participants.

[2]Use the category that best describes the ethnic/racial status for all U.S. Citizens and Non-citizens with Permanent Residency. *(If more than one category applies, use the one category that most closely reflects the person's recognition in the community.)*

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AMERICAN INDIAN OR ALASKAN NATIVE: A person having origins in any of the original peoples of North America, and who maintain cultural identification through tribal affiliation or community recognition.

ASIAN: A person having origins in any of the original peoples of East Asia, Southeast Asia and the Indian subcontinent. This area includes, for example, China, India, Indonesia, Japan, Korea and Vietnam.

BLACK, NOT OF HISPANIC ORIGIN: A person having origins in any of the black racial groups of Africa.

HISPANIC: A person of Mexican, Puerto Rican, Cuban, Central or South American or other Spanish culture or origin, regardless of race.

PACIFIC ISLANDER: A person having origins in any of the original peoples of Hawaii; the U.S. Pacific Territories of Guam, American Samoa, or the Northern Marianas; the U.S. Trust Territory of Palau; the islands of Micronesia or Melanesia; or the Philippines.

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THIS PART WILL BE PHYSICALLY SEPARATED FROM THE FINAL PROJECT REPORT AND USED AS A COMPUTER SOURCE DOCUMENT. DO NOT DUPLICATE IT ON THE REVERSE OF ANY OTHER PART OF THE FINAL REPORT.

CBT-8505960 R. S. Roberts

PART II - SUMMARY OF COMPLETED PROJECT

In preliminary experiments using high temperature and short residence times, buffered solvent pulping was found to give excellent delignification as well as a high quality pulp. Compared to current commercial pulping processes, buffered solvent pulping has a number of potential advantages which include: the pulping liquor does not contain sulfur compounds; high pulp yields are obtained; and a high quality lignin by-product can be recovered.

A series of batch pulping experiments were performed to investigate buffered solvent pulping. In particular these experiments were designed to better understand delignification of tulip poplar wood in an essentially neutral mixture of ethanol and water.

For temperatures between 200 and 240°C, delignification was found to proceed first in a relatively fast phase which is then followed by a relatively slow phase. Buffered solvent delignification for both the relatively fast phase and the relatively slow phase can be modelled as first order with respect to lignin. Quantitative ^{13}C NMR was used to characterize the solubilized lignin. The solubilized lignin was relatively uncondensed with few carbohydrates associated with it. Solubilized syringyl lignin removed first was more etherified than syringyl lignin removed later.

The pulp obtained in buffered solvent pulping of tulip poplar was found to excellent properties. In spite of the high temperatures, high viscosity pulps were obtained indicating the cellulose retains a high degree of polymerization. X-ray diffraction spectrometry indicated the cellulose in the pulp retained a structure which is characteristic of native crystalline cellulose. The pulp is an excellent feed stock for the enzymatic production of fermentable sugars.

CBT-8505960 R. S. Roberts

PART III - TECHNICAL INFORMATION

Publications resulting from Grant CBT-8505960

A. Referred Publications*

1. G. S. Faass, R. S. Roberts, and J. D. Muzzy, "Buffered Solvent Pulping," **Holzforschung**, **43**, 245-250 (1989).

2. M. F. DeLange, J. A. Trummer, R. S. Roberts, J. D. Muzzy, and L. T. Gelbaum, "^{13}C NMR Characterization of Tulip Poplar Lignin Solubilized by Buffered Solvent Pulping," in press **Holzforschung**.

3. H. M. Kitsos, R. S. Roberts, and J. D. Muzzy, "N-Propylamine Pretreatment of Pulp to Enhance Enzymatic Hydrolysis," in press **Bioresource Technology**.

*Note reprints of publication 1 are included in the appendix. A copy of the Galley for publication 2 and a copy of the accepted manuscript for publication 3 are also included in the appendix. Reprints of publications 2 and 3 will be forwarded after they are received from the publishers.

B. Conference Presentations

1. J. A. Trummer, R. S. Roberts, and J. D. Muzzy, "Buffered Solvent Delignification," presented at the 1987 National Summer Meeting of the American Institute of Chemical Engineers, Minneapolis, MN, August 1987.

2. M. F. DeLange, J. A. Trummer, R. S. Roberts, J.D. Muzzy, and L.T. Gelbaum, "^{13}C NMR Characterization of Buffered Solvent Pulped Lignins," presented at the 1990 Spring National Meeting of the American Institute of Chemical Engineers, Orlando, FL, March 22-28, 1990.

3. K. Driscoll, J. A. Trummer, R. S. Roberts, and J. D. Muzzy, "Bulk and Residual Delignification in Buffered Solvent Pulping," presented at the 1990 Annual Meeting of the American Institute of Chemical Engineers, Chicago, IL, November 11-16, 1990.

C. Thesis

1. J. A. Trummer, "Characterization of Lignin Reactions in Buffered Solvent Pulping," Master of Science in Chemical Engineering Thesis, Georgia Institute of Technology, June 1987.

2. M. F. DeLange, "Quantitative ^{13}C NMR of Poplar Lignins Obtained from Neutral Solvent Pulping," Master of Science in Chemical Engineering Thesis, Georgia Institute of Technology, June 1989.

DISCUSSION

The data and the analysis of the data are discussed in the manuscripts located in the appendices.

APPENDIX A

Holzforschung
43 (1989) 245–250

OFFPRINT

Buffered Solvent Pulping

By G.S. Faass[1)], R.S. Roberts[2)], and J.D. Muzzy

School of Chemical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332-0100, U.S.A.

Keywords

Solvent pulping
Delignification rate constants
High temperature pulping
Crystallinity
Tulip poplar

Summary

Organosolv pulping tulip poplar under approximately neutral conditions was investigated. A sodium bicarbonate buffer was used to maintain the ethanol-water-MAQ pulping liquor at relatively neutral pH. Relatively high cook temperatures (200 to 240°C) with low residence times (less than 20 minutes at temperature) were found to give excellent delignification as well as a high quality pulp.

Introduction

The economical fractionation of lignocellulosic materials continues to be one of the most vexing technical problems engineers and scientists have faced. A number of commercial fractionation processes have been developed to separate lignin from cellulose and hemicellulose. Unfortunately, the commercial processes have a number of limitations; hence, substantial research efforts have been undertaken to improve current processes or to develop new processes. Of the potential new processes, solvent pulping has been the most intensively investigated.

Early studies of solvent, or organosolv pulping, were conducted by Aronovsky (Aronovsky 1936; Aronovsky and Gortner 1936). Aronovsky discovered that a liquor composed of an organic solvent and water can be used at high temperatures to pulp biomass. He also found that delignification proceeded best in acidic solvent pulping liquors and that the pulping liquor would naturally become acidic during pulping due to the release of organic acids from wood.

Since these initial discoveries, a number of researchers have investigated organosolv pulping (Sarkanen 1980; April *et al.* 1979; Kleinert 1975; Sanwal 1978; Young *et al.* 1987). These investigations have all studied organosolv pulping using either acidic or basic pulping liquors. Acidic or basic pulping liquors have been historically used in the kraft, soda, and neutral sulfite pulping processes to promote lignin fragmentation and solvation. However, these acids or bases also promote undesirable side reactions, such as lignin condensation and depolymerization of hemicellulose/cellulose (Chua and Wayman 1979; Gierer 1970, 1982; Muzzy *et al.* 1983). The acids and bases present in pulping liquors are non-specific catalysts since they promote both desirable and undesirable reactions. Remarkably, the desirability of these non-specific catalysts has not been extensively explored for organosolv pulping. Recently an organosolv process which does not utilize these non-specific catalysts for pulping has been investigated at Georgia Tech (Faass *et al.* 1984; Faass *et al.* 1985).

In the process being developed at Georgia Tech, a buffer is used to maintain a relatively neutral pH. Since the pulping takes place at a relatively neutral pH, comparably high temperatures can be utilized without damaging the pulp. Lignin fragmentation and solvation is promoted by the high temperature and an organic lignin solvent (ethanol-water). Methylanthraquinone (MAQ) is added to the pulping liquor to reduce lignin condensation reactions and to stabilize the carbohydrate pulp. In this paper, the results of pulping under buffered solvent conditions are reported.

Materials and Methods

Pulping operations

Fresh chipped tulip poplar *(Lirodendron tulipfera)* was obtained from a local chipping operation. For all experiments except where noted, the chips were reduced to a nominal 1 mm by Wiley milling and refrigerated. Pulping cooks were performed using a 650 ml stirred batch reactor manufactured by Pharr Instruments, Inc. The protocol for each cook involved degassing the wood followed by soaking the wood in a pulping liquor composed of ethanol, water, sodium bicarbonate, and methylanthraquinone, as shown in Table 1.

Table 1. Components for MAQ-neutral solvent pulping experiment

Ethanol	180 ml
Water	120 ml
Sodium Bicarbonate	7 g
Wood (dry basis)	33 g
MAQ	1.2 g

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[2)] To whom correspondence should be addressed.

The reactants were charged into the reactor, heated to the desired temperature (typically within 30 minutes), held for the required cook time, and then cooled. The products were filtered, washed and dried to obtain pulp yield.

Lignin analysis

Lignin determinations were made using TAPPI Standard T222 os-74 and T236 os-76 methods.

Carbohydrate analysis

A Waters GPC I with a Biorad HPX-87P column was used for carbohydrate analysis. Samples were prepared first by adding 3 ml of 72 percent sulfuric acid to 0.35 g of wood and stirring. After one hour for primary hydrolysis, the sample was diluted with 84 ml of water and autoclaved at 15 psig for one hour. After cooling, the lactose internal standard was added and a 10 ml aliquot of the solution was removed. The aliquot was neutralized with lead carbonate and centrifuged to remove the precipitate. The supernatant was filtered using a C18 Sep-pak cartridge manufactured by Waters before being injected into the HPLC.

Pulp viscosity

The pulp viscosities were determined by using a modified TAPPI Standard Method T230 os-76. An Appropriate Cannon-Fenske capillary viscometer in a 25°C water bath was used to measure the viscosities.

X-ray diffraction spectrometry

A Siemens 90-degree X-ray Diffractometer was employed to obtain the diffraction tracings. Samples were prepared to pass through a 40-mesh screen and then 0.15 g of the material was compressed into an aluminum sample holder under a pressure of 1000 psia to form a disc of 5/8 inches in diameter. The sample was then mounted in the diffractometer and was scanned under the following conditions:

Scanning Range 2θ	10 to 30 degrees
Scanning Step	0.1 degree
Exposure Time	120 s/0.1 degree

Results and Discussion

Delignification

In an initial series of experiments, called the non-isothermal cooks, the reactor was rapidly heated to temperatures ranging from 100 to 280°C and quickly cooled. A maximum of 32 minutes was required to heat the reactor to the experimental temperature; while a maximum of 12 minutes was required to cool the reactor. The results of the non-isothermal cooks are shown in Figure 1. As can be seen, delignification essentially does not occur until the temperature exceeds 160°C and very little delignification occurs until the temperature exceeds 180°C. Initially the pulping liquor was at a pH of approximately 9.5 and essentially remained unchanged until the temperature of the pulping liquor exceeded 160°C. When the temperature of the pulping liquor reached 180°C, the pH of the liquor was approximately 7.2. Hence essentially all of the delignification above 180°C took place under approximately neutral conditions.

The non-isothermal cooks were augmented by additional cooks called the isothermal cooks. In these

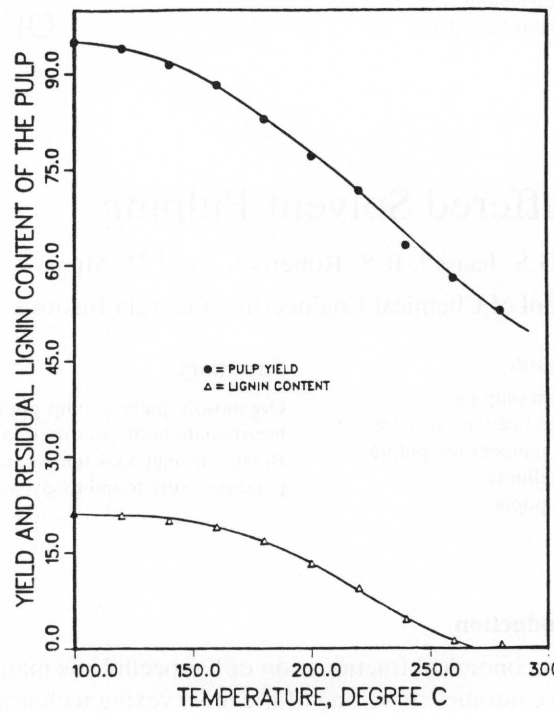

Fig. 1. Yield and residual lignin content of pulps obtained during the non-isothermal stage of the cook

cooks, the reactor was heated to a specific temperature ranging from 200 to 260°C and held at that temperature for up to 45 minutes. As can be seen in Figure 2, delignification in the isothermal portions of the 200, 220 and 240°C cooks are characterized by an ini-

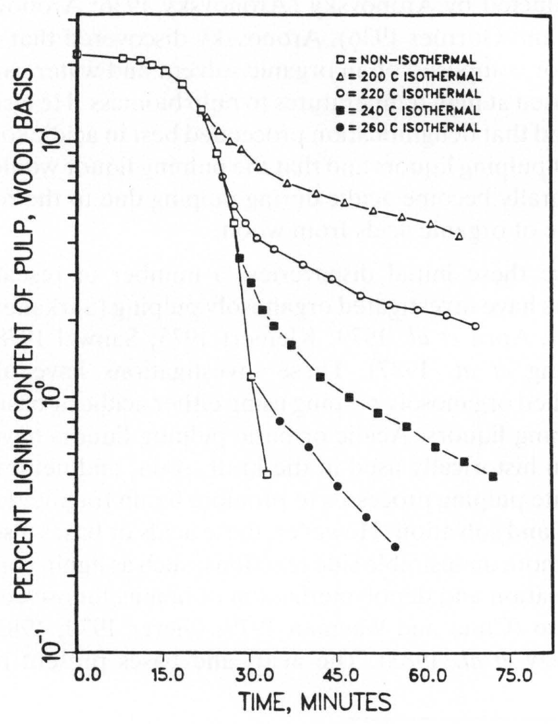

Fig. 2. Experimental delignification data

tial "fast" step followed by a second "slow" step. In the case of the 260°C cooks, only one stage of delignification was observed; however, the lignin content of the wood at the beginning of the isothermal cook was very low. As shown in Figure 2, for the latter portions of the 200, 220, 240, and 260°C cooks the logarithm of the lignin content appears to be directly proportional to the time at temperature.

Linear regressions were performed on these cooks and the best straight line fit of the data was obtained, as well as the correlation coefficient, r. The results are shown in Table 2. In each case the correlation of the data to a straight line is very high as indicated by the absolute value of r being near unity. These results verify that this stage of delignification can be suitably modeled as a first order process. Using the data shown in Table 2, the rate constants were calculated assuming an Arrhenius relationship (Fig. 3). The activation energy and frequency factor were 13.46 kcal/gmole and $1.85 \cdot 10^4$ min^{-1}, respectively.

Table 2. First order rate constants stage of delignification for the slow

Temperature °C	Rate Constant $minutes^{-1}$	Correlation Coefficient, r
200	0.012	−0.957
220	0.019	−0.941
240	0.033	−0.968
260	0.058	−0.980

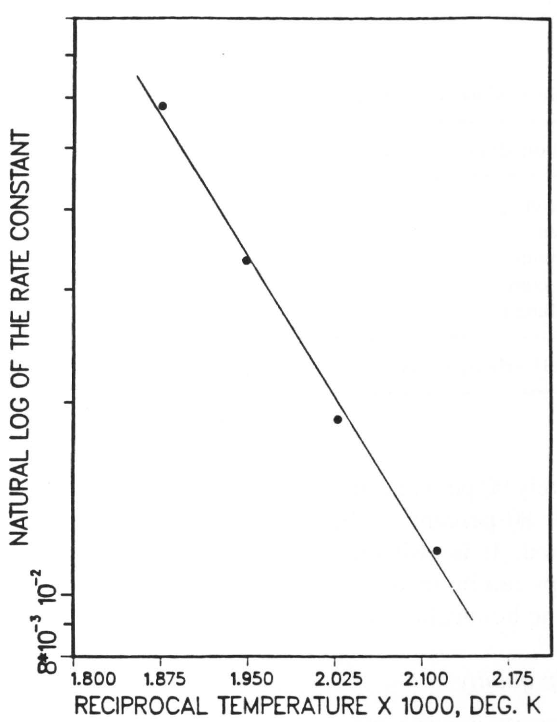

Fig. 3. Arrhenius plot for the slow stage of delignification

Table 3. First order rate constants for the rapid stage of delignification

Temperature °C	Rate Constant $minutes^{-1}$	Correlation Coefficient, r
200	0.126	−0.997
220	0.224	−0.953
240	0.399	−0.989

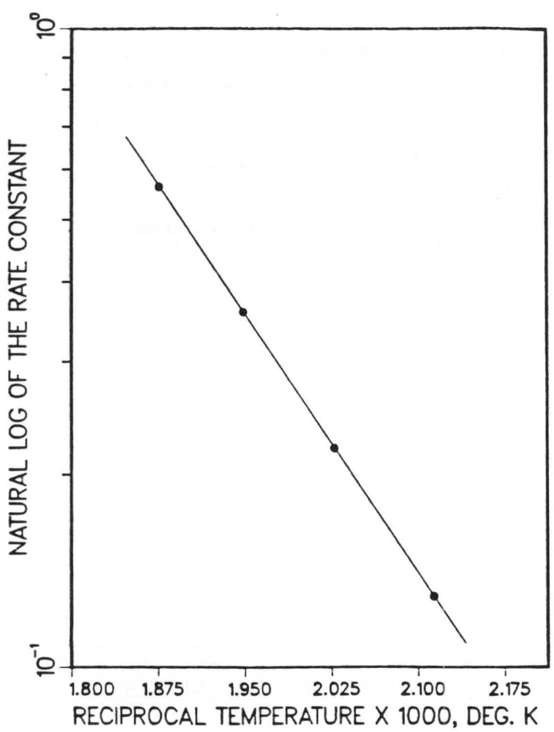

Fig. 4. Arrhenius plot for the rapid stage of delignification

Analyzing the "fast" delignification step is more complex than analyzing the "slow" delignification step. The effects of the "slow" delignification must be subtracted from the data in order to determine the true fast delignification rates. After compensation for the "slow" delignification, regression analysis indicated the true "fast" delignification would best be described as a first order process, a finding which is in agreement with the literature (Kleinert 1966A; 1966B). The first order rate constants and correlation coefficients are shown in Table 3. An Arrhenius plot was made (see Fig. 4) to determine the activation energy and the frequency factor, 13.88 kcal/gmole and $3.26 \cdot 10^5$ $minutes^{-1}$ respectively.

Pulp yields

The effects of buffered solvent pulping on the pulp were also determined. Isothermal carbohydrate yields are shown in Figures 5, 6 and 7 while the corresponding steady state isothermal rates of carbohydrate loss are given in Table 4. The initial composition of the tulip poplar is given in Table 5. As indicated by the figures and tables, removal of the non-specific catalysts (acids and bases) results in the need for higher pulping temperatures than those used in conventional pulping processes. At these relatively high temperatures and short cook times, the buffered solvent process gives superior fractionation of the lignocellulosic material. For example for a 220°C/20 minutes cook, approximately 90 per cent of the lignin can be removed while over 80 percent of the carbohydrate pulp can be retained. It is also important to note that low lignin pulps can be made while substantially retaining most of the hemicellulose.

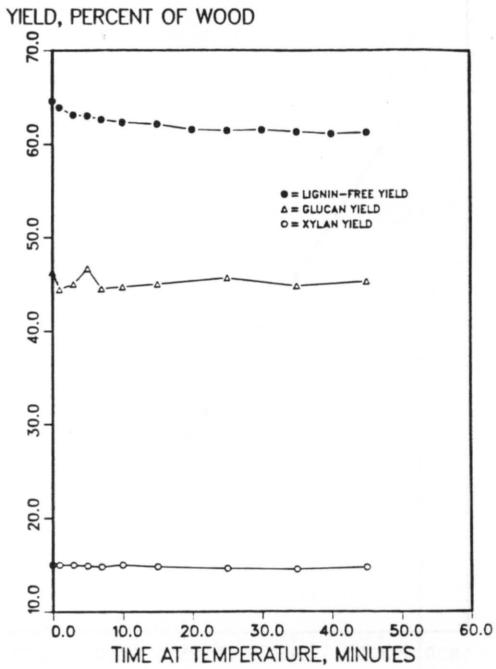

Fig. 5. 200°C Isothermal carbohydrate yield data

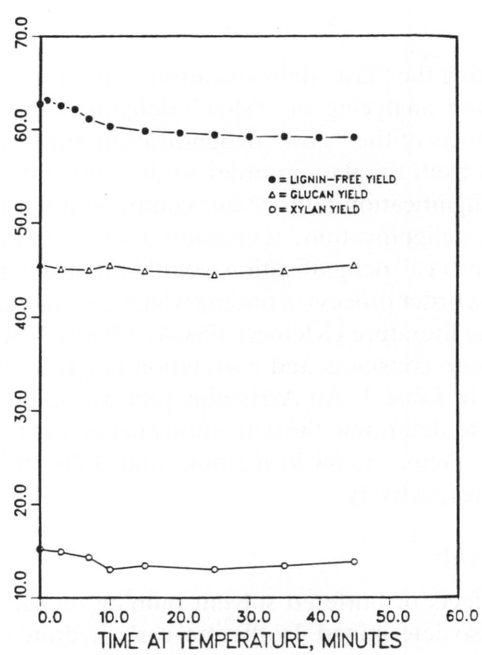

Fig. 6. 220°C Isothermal carbohydrate yield data

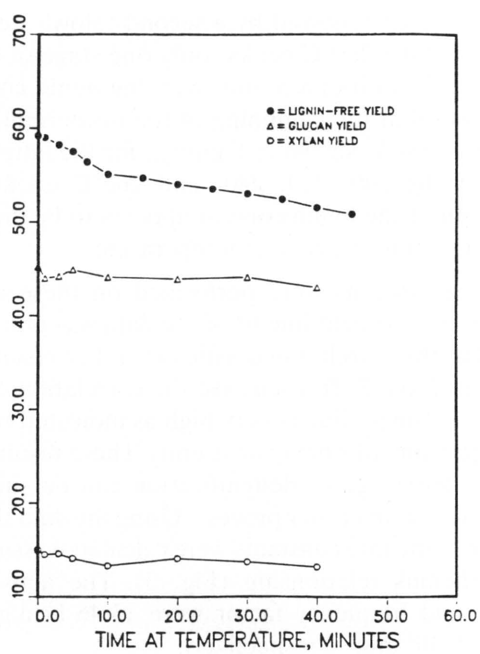

Fig. 7. 240°C Isothermal carbohydrate yield data

Table 4. Isothermal rates of carbohydrate removal

Temperature °C	Rate Based on Lignin-Free Yield g of Carbohydrate / 100 g Wood min	Rate Based on Cumulative Carbohydrate Loss g of Carbohydrate / 100 g Wood min
200	0.05	0.03
220	0.10	0.04
240	0.17	0.10
260	0.35	–

Table 5. Carbohydrate analysis of tulip poplar

Carbohydrates	Wt Percent Wood
Glucan	49.5
Xylan	17.2
Mannan	3.7
Galactan	0.98
Arabinan	0.69
Total Carbohydrates	72.1

Pulp quality

Viscosity measurements were performed on samples prepared during the isothermal delignification study.

Table 6. Influence of reaction conditions on the 0.5% TAPPI CED viscosities of pulps using a buffered ethanol-water solvent system containing 4% MAQ

Condition of the Wood	Milled					Chips				
Maximum Cooking Temperature	220	220	240	240	260	220	220	240	240	260
Time at Maximum Cooking Temperature	20	40	5	20	5	20	40	5	20	5
.5% TAPPI CED Viscosity (KPa.s)	26.3	29.2	17.8	12.4	5.9	31.1	46.9	27.5	27.5	10.8

Table 7. Physical properties of bleached buffered solvent pulp

Sample	Brightness %	Opacity %	Tensile lbs/in	Tear g	Mullen psig	Density g/cm³
Bleached Buffered Solvent Pulp	86.0	75.9	10.1	42.4	12.3	0.52
Typical Mixed Hardwood Pulp*	91.9	76.1	12.0	45.5	11.0	0.57

* Obtained from Nackawic Pulp and Paper Company, Ltd.

Even though the pulps had been prepared from milled wood the resulting viscosities were surprisingly high as shown in Table 6. As expected the viscosities tended to decrease as the reaction temperature increased. This trend was further supported by data made from cooks using chips in place of milled wood (see Table 6). As expected, pulps made from chips had consistently higher viscosities than those made from milled wood.

Having obtained these viscosity data, a 230 °C, 15 minute cook was made using chips. This sample was sent to a major pulp company for subsequent physical property analysis. The TAPPI viscosity of the unbleached and bleached pulp were 40.3 and 10.9 cps, respectively. Selected physical properties of handsheets made from the unbeaten bleached pulp were obtained and are summarized in Table 7.

Also shown in Table 7 for comparison are data for a typical mixed hardwood kraft pulp containing 13 percent softwood. These data were provided by Nackawic Pulp and Paper Company, Ltd. The pulp was bleached, unbeaten and had a viscosity of 18.4 cps. From these very limited data, the properties of the buffered solvent pulp appear to be comparable with the kraft pulp. Detailed studies on the physical properties of buffered solvent pulp to confirm these preliminary results are currently being conducted.

To determine the effect of high temperature buffered solvent pulping on the crystallinity of the pulp, X-ray diffractograms were made of the milled wood, the 220 °C/20 minute pulp, and the 240 °C/0 minute pulp. These diffractograms are shown in Figures 8, 9 and 10. The diffractogram of the milled wood is very noisy due to the high lignin content of the untreated wood.

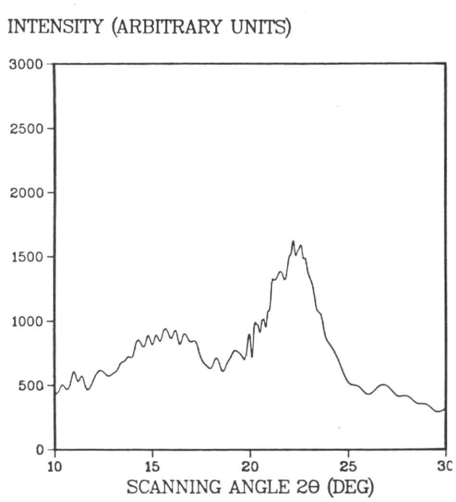

Fig. 8. X-Ray diffractogram of milled poplar wood-40 mesh

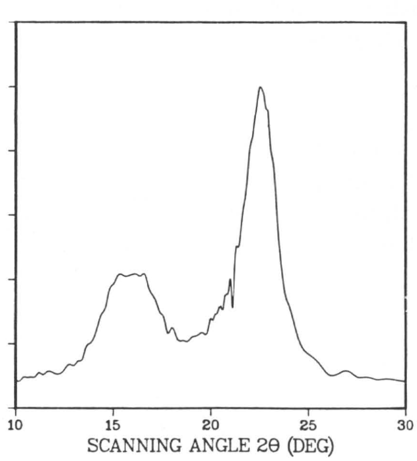

Fig. 9. X-Ray diffractogram of pulp 220°C/20 min

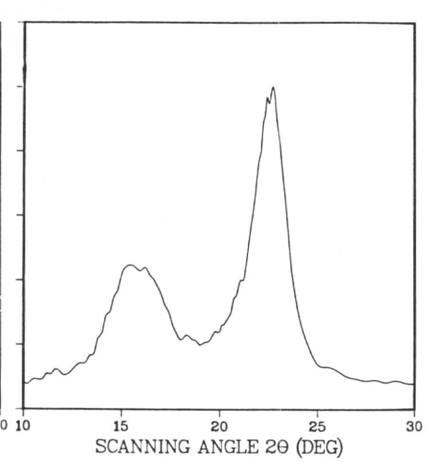

Fig. 10. X-Ray diffractogram of pulp 240°C/0 min

In contrast the diffractograms of the pulps have strong and relatively sharp peaks which are characteristic of native cellulose I. Both of the samples are remarkably similar in terms of their crystalline structure. It appears that the high temperature buffered solvent delignification process efficiently preserves the native cellulose crystalline structure.

Process economic considerations

For buffered solvent pulping to be economically feasible, the commercial process would of course be significantly different from the bench scale experiments described previously. The digester would be a continuous-type operating with a wood to liquor ratio of approximately 1 to 3 (weight to volume). The pH of the pulping liquor would be maintained at close to neutrality by the controlled addition of sodium carbonate instead of the sodium bicarbonate used in the bench scale experiments. Economics also dictate that essentially all of the pulping liquor chemicals be recovered and recycled.

Recovery of the ethanol, sodium carbonate, and MAQ pulping chemicals can be accomplished using standard technology. Preliminary design calculations indicate that the MAQ could be recovered by liquid-liquid extraction and ethanol can be recovered by fractional distillation. After removal of ethanol from the pulping liquor, by-product lignin compounds would precipitate and be separated from the water soluble sodium compounds by filtration. The non-volatile compounds remaining in the aqueous solution would be concentrated, combusted, and the sodium carbonate recovered by aqueous leaching of the combustion ash.

With only the preliminary data currently available, process economics for a commercial buffered solvent pulping facility is difficult to assess. Preliminary economic analysis based on conservative design assumptions does however indicate the fixed capital investment and operating costs of such a facility are favorable when compared to those of a kraft pulp mill.

Conclusions

Organosolv pulping liquor does not require the presence of acids or bases to delignify angiospermous wood. The use of relatively neutral pulping liquors allows the wood to be pulped at high temperatures for short residence times without significantly damaging the carbohydrate pulp. A high degree of delignification can be obtained while retaining substantially all of the carbohydrate fraction in the pulp.

Acknowledgments

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References

April, G.C., M.M. Kamal, J.A. Reddy, G.H. Bowers and S.M. Hansen. 1979. Delignification with Aqueous-Organic Solvents. Tappi *62* (5): 83–85.

Aronovsky, S.I. 1936. Alcoholic Treatment of Ligneous Cellulosic Material. U.S. Patent 2,037,001.

Aronovsky, S.I., and R.A. Gortner. 1936. Cook Process IX. Pulping Woods with Alcohols and Other Organic Reagents. Ind. Eng. Chem. *28*, 1270–1276.

Chua, M.G.S., and M. Wayman. 1979. Characterization of Autohydrolysis Aspen Lignins. Part 1. Composition and Molecular Weight Distribution of Extracted Autohydrolysis Lignin. Can. J. Chem. *57*, 1142–1149.

Faass, G.S., J.D. Muzzy, R.S. Roberts and M.A. Bonnano. 1984. Accelerated Solvent Pulping Process Development. Presented at the 1984 Annual Meeting AIChE, San Francisco, CA.

Faass, G.S., R.S. Roberts and J.D. Muzzy. 1985. Modelling MAQ-Neutral Solvent Delignification. Presented at the 1985 Summer National Meeting AIChE, Seattle, WA.

Gierer, J. 1970. The Reactions of Lignin During Pulping. Svensk Papperstidn. *73*, 18, 571–596.

Gierer, J. 1982. The Chemistry of Delignification. Holzforschung *36*, 43–51; 55–64.

Kleinert, T.N. 1966A. Mechanisms of Alkaline Delignification I. The Overall Reaction Pattern. Tappi *49* (2): 53–57.

Kleinert, T.N. 1966B. Mechanisms of Alkaline Delignification II. Free Radical Reactions. Tappi *49* (3): 126–130.

Kleinert, T.N. 1975. Ethanol-Water Delignification of Wood-Rate Constants and Activation Energy. Tappi *58* (8): 170–171.

Muzzy, J.D., R.S. Roberts, C.A. Fieber, G.S. Faass and T.M. Mann. 1983. Pretreatment of Hardwood by Continuous Steam Hydrolysis. *In:* "Wood and Agricultural Residue" (E.J. Soltes, Ed.) Academic Press. New York. p. 351–368.

Sanwal, J.S. 1978. Organosolv Pulping of Red Alder and Wheat Straw. M.S. Thesis. University of Washington.

Sarkanen, K.V. 1980. Acid-Catalyzed Delignification of Lignocellulosics in Organic Solvents. *In:* "Progress in Biomass Conversion." Vol. 2. (K.V. Sarkanen and D.A. Tillman, Eds.). Academic Press. New York. p. 127–144.

Young, R.A. 1987. Ester Pulping. Presented at the Lake States TAPPI Meeting on "Pulping and Deinking", Wausau, WI, April 1987.

APPENDIX B

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^{13}C NMR Characterization of Tulip Poplar Lignin Solubilized by Buffered Solvent Pulping

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Keywords
^{13}C NMR
Lignin
Milled wood lignin
Organosolv pulping
Tulip poplar
Liriodendron tulipifera

Summary

^{13}C NMR Spectroscopy was used to study the mechanism of buffered solvent pulping. Solubilized lignin was relatively uncondensed with few carbohydrates associated with it. Solubilized syringyl lignin removed first was more etherified than syringyl lignin removed later. Quantitative NMR data was required to obtain meaningful results.

Introduction

Buffered solvent pulping (Faass *et al.* 1989) is a unique organosolv process for fractionating lignocellulosic materials. The unique feature about this process is that it delignifies wood at neutral pH using a 50% aqueous ethanol liquor buffered with sodium bicarbonate. By pulping at neutral pH, lignin condensation reactions and depolymerization of carbohydrates are minimized. By eliminating acid or base catalysts, temperatures in the range 200 to 240 degree Celsius are required to obtain low lignin contents in the pulp. Despite the high temperatures, lignin free pulp yields greater than 60% with strength properties comparable to Kraft pulps are obtained.

Faass (1989) modelled the buffered solvent pulping as two first order processes occurring simultaneously. The bulk delignification process was most significant early in the cook, especially during the nonisothermal heat up of the reactor. Upon reaching the desired pulping temperature, bulk delignification gradually became less significant until only residual delignification was observed.

This study reports on the mechanism of buffered solvent pulping. It is generally accepted that acid catalyzed organosolv pulping does not cleave the predominant β−O−4 ether linkages in lignin (Sarkanen 1980). This was based on activation energy studies. Sarkanen reported an activation energy of 8.4 kcal/mole for an organosolv process employing an acid catalyst. The activation energy required to cleave β−O−4 linkages in model compounds was 36 kcal/mole. Sarkanen concluded that the probable mechanism of orgranosolv pulping was cleavage of α aryl ether linkages which were less stable than β−O−4 ether linkages. The resulting reduction in the lignin molecular weight facilitated its solubilization in the pulping liquor.

The activation energy of buffered solvent pulping was determined to be 13.9 kcal/mole (Faass *et al.* 1989). This was much less than 36 kcal/mole activation energy reported by Sarkanen for cleavage of β−O−4 model compounds. Therefore, one would not expect cleavage of β−O−4 ether linkages to be significant in buffered solvent pulping.

Quantitative ^{13}C NMR was used to study the structure of lignin solubilized by bufferd solvent pulping. Differences observed in the structure of buffered solvent lignin compared to milled wood lignin provided insights to the mechanism of buffered solvent pulping.

Materials and Methods

Lignin sample collection

Tulip poplar (*Liriodendron tulipfera*) was milled to a nominal 1 mm diameter and refrigerated. Pulping cooks were performed using a 650 ml stirred batch reactor manufactured by Parr Instruments, Inc as described by Faass *et al.* (1989) except methylanthraquinone was omitted. The protocol is shown schematically in Figure 1. The 190/0 lignin represents lignin removed by bulk delignification during the time when most of the acid formation and neutralization occur. The 220/0 lignin represents lignin removed by bulk delignification after the time when most of the acid formation and neutralization took place. The 220/15 lignin represents lignin removed by both bulk and residual delignification. The 220/60 lignin represents lignin removed by residual delignification.

Milled wood lignin was obtained per Björkman (1956).

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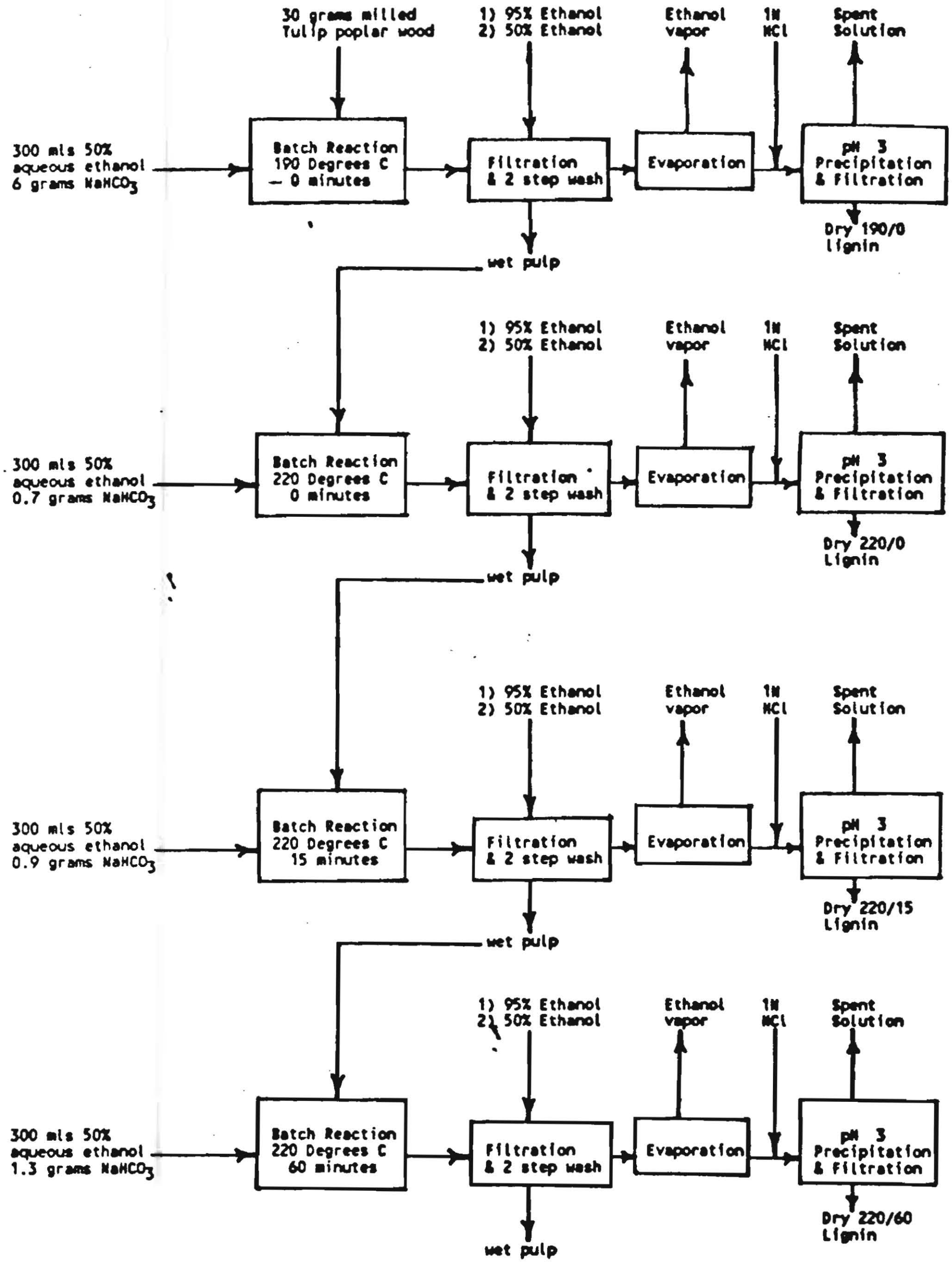

Note: 1) Batch Temperature refers to the maximum temperature attained.
2) The reactor was cooled to room temperature after being held at the maximum temperature for the stated time.

Fig. 1. Lignin sample collection protocol

NMR analysis

NMR samples were prepared by dissolving approximately 300 mg of vacuum dried lignin in 4.5 mls of deutrated dimethylsulphoxide (DMSO) which contained dioxane as an internal standard.

Proton decoupled ^{13}C NMR spectra were recorded for the lignin samples at room temperature using a Varian XL-400 spectrometer operating at 100.575 MHz. The DMSO provided the internal deuterium lock. The center line of DMSO provided the reference line at 39.5 ppm. Waltz 16 decoupling was used (Shaka *et al.* 1983). Quantitative spectra were performed as recommended by Landucci (1985). One deviation from Landucci was that a 6 second delay time was used instead of the recommended 8 second relaxation delay time. A comparison of a spectrum performed with a 6.0 second delay time to one with an 8 and 12 second delay time showed no differences for the peaks of interest (i.e. those representing carbons 2, 3, 5 and 6 of syringyl lignin and carbons 2, 5 and 6 of guaiacyl lignin). Other parameters used for obtaining the quantitative spectra are listed in Table 1.

Table 1. Quantitative NMR Parameters

Number of scans per sample	10000
Pulse width (micro seconds)	12
Pulse angle (degrees)	45
Acquisition time (seconds)	0.4
Relaxation delay time (seconds)	5.6
Spectral width (ppm)	200
Number of data points	16000
Digital resolution (Hertz)	1.2

The FID data was Fourier transformed from the time domain to the frequency domain, phased, and written to aSCII files using the FTNMR software from Hare Research Inc. The peaks were integrated using SPECTRA CALC software from Galactic Industries Corporation using a personal. computer.

Results and Discussion

General observations

Quantitative ^{13}C NMR spectra of lignin samples MWL, 190/0, 220/0, 220/15 and 220/60 are presented in Figure 2. Table 2 lists the peak assignments based on assignments reported in the literature (Nimz *et al.* 1981; Marchessault *et al.* 1982; Lapierre *et al.* 1982; Nimz *et al.* 1982; Lapierre 1984, Bardet *et al.* 1985; Trummer 1987; Evliya 1989). The milled wood lignin sample, MWL, represented lignin in its native state.

The large peak at 40 ppm in the spectra was from the DMSO solvent. The peak at 66 ppm is the dioxane reference peak.

Table 2. Peak Assignments for Figure 2

Region	ppm Range	Assignment
1	151–155	Carbons 3 and 5 of etherified syringyl
2	145–150	Carbons 3 and 5 of phenolic syringyl Carbons 3 and 4 Gualacyl
3	137–140	Carbon 4 of etherified syringyl
4	133–136	Carbon 1 of guaiacyl and syringyl Carbon 4 of phenolic syringyl
5	118–121	Carbon 6 of guaiacyl
6	114–117	Carbon 5 of guaiacyl
7	110–113	Carbon 2 of guaiacyl
8	102–108	Carbons 2 and 6 of syringyl
9	84– 88	β carbons of β–O–4 and α carbons of phenolcoumarans and pinoresinol
10	71– 75	α carbons of β–O–4 and γ carbons of pinoresinol
11	58– 61	γ carbons of β–O–4 and phenylcoumarans
12	55– 57	Methoxy groups
13	54	β carbons of phenolcoumarans and pinoresinol
	20, 63, 75– 83, 96–102	Carbohydrates – mostly xylans
	25– 35	Saturated α, β, γ carbons associated with condensation products
	170	Carbonyl of carboxilic acids
	130	Aliphatic vinyls

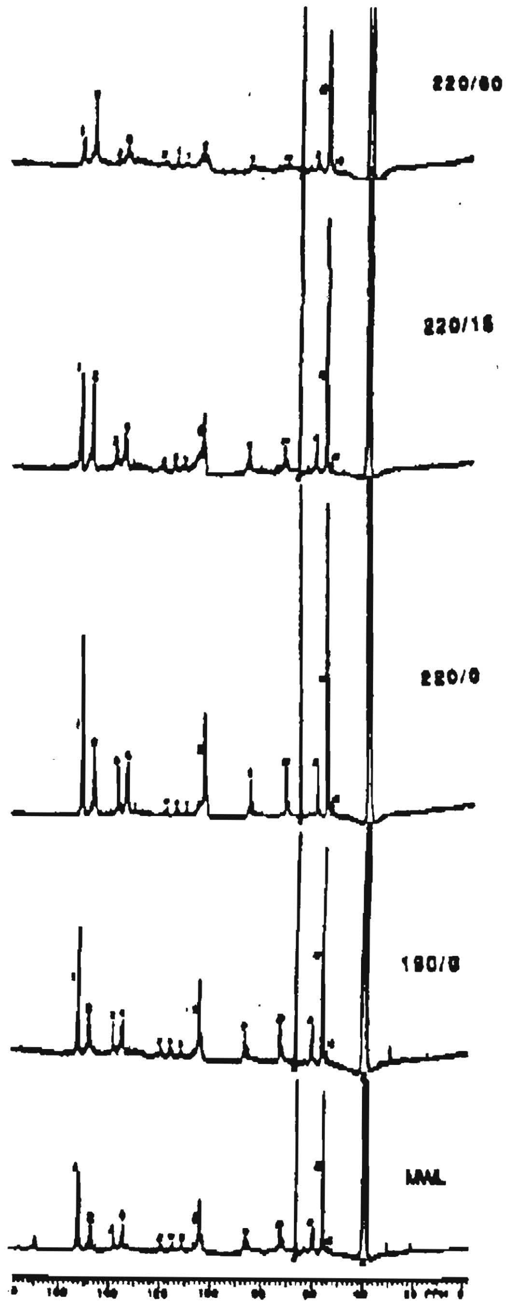

Fig. 2. Quantitative ^{13}C NMR spectra of tulip poplar milled wood, 190/0, 220/0, 220/15, and 220/60 lignins

The spectra showed that lignin solubilized by neutral solvent pulping had few carbohydrates associated with them from the lack of carbohydrate peaks at 20, 63, 75–83 and 96–102 ppm. This was in agreement with Sarkanen's (1980) study of acid catalyzed organosolv pulping. The carbohydrate peaks were present in the milled wood lignin spectrum (see Fig. 2).

Carbonyls at 170 ppm were present in the milled wood lignin spectra but not the other spectra. Carbonyls

3B

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would be associated with carbohydrate degradation products from peeling reactions.

Saturated aliphatics at 30 to 50 ppm were observed in all the solubilized spectra but they are more prominent in the 190/0 spectra. Saturated aliphatics were associated with lignin polymerizing with itself and other compounds. The substances obtained were referred to as condensation products.

The spectra indicated that most of the condensation products occurred early in neutral solvent pulping. Goldstein (1985) concluded that lignins remain uncondensed in the residual pulp. His conclusion is substantiated since few condensation products appear late in the pulping run.

Overall area integration results

The overall areas for the normalized spectra for the quantitative spectra are shown in Figure 3A. The overall areas of the quantitative spectra were relatively constant even though the areas of individual peaks vary in the spectra. This implies that the ratio of area per number of carbon atoms is constant through out the quantitative spectra. It is possible to directly compare the relative quantities of carbon bonds in a lignin molecule.

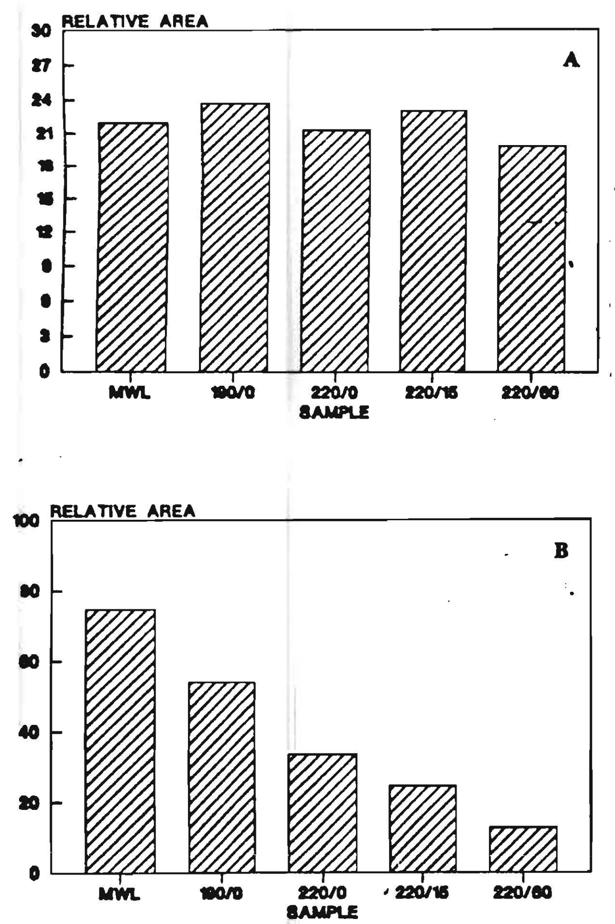

Fig. 3. **A.** Total relative ^{13}C area of normalized quantitative spectra **B.** Percent syringyl etherification of the samples

Overall area integration results

The overall areas for the normalized spectra for the quantitative spectra are shown in Figure 3A. The overall areas of the quantitative spectra were relatively constant even though the areas of individual peaks vary in the spectra. This implies that the ratio of area per number of carbon atoms is constant through out the quantitative spectra. It is possible to directly compare the relative quantities of carbon bonds in a lignin molecule.

β−O−4 ether linkage cleavage

Figure 3B shows the percent syringyl etherification of the samples based on data obtained from the quantitative spectra. The values were calculated using the quantitative normalized integrated areas of regions 1, 2, and the average of regions 5, 6, and 7. Region 1 represents all the etherified syringyl carbons 3 and 5. Region 2 represents all the phenolic syringyl carbons 3 and 5 as well as guaiacyl carbons 3 and 4. It is difficult to determine the contribution of carbons 3 and 4 to region 2. There can only be one of each of carbons 1 through 6 in as phenyl ring. It is possible to estimate the contribution of guaicyl carbons 3 and 4 by using regions 5, 6 and 7 which represents carbons 6, 5, and 2 repactively. The contribution of guaiacyl carbons were taken out of region 2 by substracting from it twice the average of regions 5, 6 and 7.

Figure 3B shows the percent syringyl etherification decreased as the neutral solvent pulping run proceeded. The decrease could be accounted for by cleavage of α−O−4 linkages. This supports Sarkanen's conclusion that β−O−4 linkages are not cleaved in organosolv pulping (Sarkanen 1980).

Conclusions

The soluble syringyl lignin obtained during neutral solvent pulping had increased phenolic contents along with decreased etherified contents which indicate that ether linkages are cleaved as pulping proceeds. The data show that lignin removed in later stages of delignification had more ether linkage cleavage than lignin removed in early stages. The likely mechanism for residual lignin removal is hydrolysis of lignin ether linkages to form relatively small molecules followed by solubilization and diffusion through the wood.

Acknowledgements

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References

Bardet, M., M.F. Foray and D. Robert. 1985. Use of the DEPT Pulse Sequence to Facilitate the ^{13}C NMR Structural Analysis of Lignins. Makromol. Chemistry *186*, 1495–1504.

Björkman, A. 1956. Studies on Finely Divided Wood. part I. Extraction of Lignin With Neutral Solvent. Svensk Paperstidn. *59*, 477–485.

Evliya, H. 1989. ^{13}C NMR Studies of a Dehydropolymer (DHP) from Isoeugenol; Comparison with Spruce Lignin. Holzforschung *43* (1): 61–64.

Faass, G.S., R.S. Roberts and J.D. Muzzy. 1989. Buffered Solvent Pulping. Holzforschung *43* (4): 245–250.

Goldstein, S.A. 1985. Determination of the Degree of Condensation of Lignin from Solvent Pulping and Steam Hydrolysis of Tulip Poplar, M.S. Thesis. Georgia Institute of Technology, Atlanta, Georgia.

Landucci, L.L. 1985. Quantitative Characterization of Lignin. 1. A Methodology for High Precision. Holzforschung *39* (6): 355–359.

Lapierre, C., J.Y. Lallemand and B. Monties. 1982. Evidence of Lignin Heterogeneity by Combination of ^{13}C Labelled Poplar Lignins: ^{13}C NMR Experiments. Holzforschung *38* (6): 333–342.

Marchessault, R.H., S. Coulombe and H. Morikawa. 1982. Characterization of Aspen Exploded Wood Lignin. Canadian J. Chemistry *60*, 2372–2382.

Nimz, H.H. D. Robert, O. Faix and N. Nemr. 1981. ^{13}C NMR Spectra of Lignins, 8. Holzforschung *35* (1): 16–26.

Nimz, H.H., M. Nemr, P. Schmidt, C. Margot, B. Schaub and M. Schlosser. 1982. ^{13}C NMR Spectra of Lignins 9. Spin-Lattice Relaxation Times and Determination of Interunit Linkages in Three Hardwood Lignins (*Alnus glutinosa, Corylus avellana and Acer pseudoplatanus*). J. Wood Chem. Technol. *2* (4): 371–382.

Sarkanen, K.V. 1980. Acid Catalyzed Delignification of Lignocellulosics in Organic Solvents. Progress in Biomass Conversion *2*, 128–144.

Shaka, A.J., J. Keeler, T. Frenkiet and R. Freeman. 1983. An Improved Sequence for Broad Band Decoupling-Waltz 16. J. Magn. Reson. *52* (2): 335–338.

Trummer, J.A. 1987. Characterization of Lignin Reactions in Buffered Solvent Pulping, M.S. Thesis. Georgia Institute of Technology, Atlanta, Georgia.

APPENDIX C

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School of Chemical Engineering
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January 29, 1991

Dear Dr Roberts

BIORESOURCE TECHNOLOGY

I am pleased to inform you that your paper 'N-propylamine pretreatment of...' has been accepted for publication in the above journal which is a merger of Biomass with Biological Wastes

In the near future we will be contacting you wish queries from our sub-editor.

Please address any further correspsondence concerning this paper to me.

Yours sincerely

David Winter

N-PROPYLAMINE PRETREATMENT OF BUFFERED SOLVENT PULP TO ENHANCE ENZYMATIC HYDROLYSIS

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School of Chemical Engineering
The Georgia Institute of Technology
Atlanta, Georgia 30332
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KEYWORDS: Biomass Pretreatment, Cellulose Hydrolysis, Propylamine, Enzymatic Hydrolysis, Swollen Cellulose

ABSTRACT

The enzymatic hydrolysis of n-propylamine swollen buffered solvent pulp was studied in batch reactors. The rate of hydrolysis and the conversion of n-propylamine swollen and water extracted pulp was found to be approximately 60 percent higher than non-swollen pulp. Residual n-propylamine in the swollen pulp was found to inhibit strongly the enzymatic hydrolysis of the pulp. Water extraction was found to remove completely the residual amine.

X-ray diffraction was used to characterize n-propylamine swollen buffered solvent pulp. Compared to non-swollen pulp, the swollen pulp was found to have reduced crystallinity and the remaining crystals were transformed from CELLULOSE I to CELLULOSE III.

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INTRODUCTION

Lignocellulosic biomass is an abundant, renewable resource which is composed mainly of lignin and carbohydrate polymers. The carbohydrate polymer can be enzymatically hydrolyzed into fermentable sugars. Unfortunately in the native state, the carbohydrate polymers are "encrusted" by the hydrophobic lignin and are therefore shielded from aqueous enzyme solutions. This shielding reduces the rate of enzymatic hydrolysis and also reduces the yield from biomass.

The rate of enzymatic hydrolysis for native biomass is also influenced by the crystallinity of the carbohydrate polymers in the biomass. The structure of native crystalline cellulose (called CELLULOSE I) prevents penetration of water into the interior of the crystal and is difficult to hydrolyze.[1,2]

Since both the lignin content and cellulose crystallinity of native biomass reduce enzyme effectiveness two steps are needed to properly pretreat lignocellulosic materials: delignification and decrystallization. In delignification processes, the lignin-carbohydrate chemical bonds and the three-dimensional macromolecular lignin network are hydrolyzed with subsequent lignin solubilization. The lignin solution and the insoluble pulp can then be separated by filtration.

A highly efficient process, buffered solvent pulping, has been developed to separate the lignin from the carbohydrate fraction by Faass *et al.*[3] In this process, biomass is treated in a high temperature buffered solvent for a relatively short residence time. Ninety percent of the lignin can be solubilized and extracted from the biomass while recovering over 80 percent of the carbohydrate as pulp.

After delignification, the crystallinity of the biomass pulp can be changed by swelling the cellulose crystal with a polar solvent. The extent to which the fluid penetrates depends upon the ability of the solvent to form hydrogen bonds with the hydroxyl groups of cellulose and upon the size of the solvent molecules. Polar solvents which swell crystalline cellulose include sodium hydroxide, potassium hydroxide, mineral acids, ammonia and amines.[1,4-9] Chemical swelling of native crystalline cellulose can result in the formation of amorphous cellulose or different forms of crystalline cellulose classified as CELLULOSE II, III and IV.[10,11]

Pretreatment of crystalline cellulose using n-propylamine offers several potential advantages in chemical recovery and operating conditions over other chemical pretreatments. For example, the pretreatment could be conducted at ambient temperatures - pressures while the amine could be recovered by vacuum drying the pulp. In this paper, the effects of n-propylamine pretreatment on the enzymatic hydrolysis of delignified biomass are reported.

MATERIALS AND METHODS

Materials

Tulip Poplar wood was obtained from a local lumber company (McClure Bros Lumber Co.) in the form of chips. The chips were washed, debarked and air-dried for four days. The chips were then reduced to a nominal 1 mm by Wiley milling and stored in a refrigerator.

Delignification Pretreatment

The nominal 1 mm chips were delignified according to Faass, *et al.* except that a two liter batch reactor (Parr Instruments) was used.[3] The reactor charge consisted of 99 grams of chips ($\sim$ 9% moisture) and approximately 900 ml of pulping liquor. The pulping liquor composition was 540 ml ethanol, 360 ml water, 21 g sodium bicarbonate, and 3.6 g methyl-anthraquinone. A pulping temperature of 220°C and a residence time of 20 minutes at that temperature was used.

After pulping, the pulp was disintegrated in a Waring Blender on maximum speed for approximately 3 minutes. The resulting mixture was filtered using porcelain funnels and washed with ethanol until the filtrate was clear. The solid residue was wet screened to insure uniformity and consistency of the pulp samples. The fraction passing a 40 mesh and retained by a 100 mesh screen was then washed with acetone and air-dried. Klason lignin content was determined by TAPPI standard method T236 os-76 as modified by Faass *et al.*[3] For buffered solvent pulp, the Klason lignin can also be determined using the Kappa number (Klason lignin content % = 0.155 * Kappa number).

Swelling Pretreatment

Approximately 10 g of air-dried pulp was charged into a three-necked 500 ml round bottom flask and placed under vacuum for 20 minutes. The flask was then purged with a slow stream of nitrogen for 30 minutes. Next, approximately 100 g of anhydrous liquid n-propylamine (98% minimum purity, Eastman Kodak Co.) was introduced and the temperature maintained at 0°C in an ice bath.

After soaking 24 hours, most of the propylamine was removed by vacuum evaporation (200 mmHg abs). After approximately 85% of the original n-propylamine was recovered, the sample was placed in a vacuum chamber (35 mmHg) over night. The resulting pulp was dry to the touch and contained an average of 11.1 ± 1.9% (w/w) propylamine.

Enzyme system

Lyophilized cellulase powder derived from *Trichoderma reesei* (Qm 9414) was provided by the Solar Energy Research Institute (Golden, CO) and was stored frozen. Novozyme 188 (300 Cellobiase Units per ml) was purchased from NOVO Laboratories and stored in a refrigerator.

Enzymatic Hydrolysis Experiments

The enzymatic hydrolysis experiments were conducted in 500 ml, stoppered, beakers. The enzyme/acetate buffer solution was a 0.5 M sodium acetate buffer (4.8 pH) which contained 2.5 filter paper units of cellulase from Qm 9414 and 2.0 units of Novozyme 188 per ml of solution. The solid concentration was a normal 5% (w/v) and the stirrer speed was 100 rpm for the hydrolysis experiments. The hydrolysis took place at 45°C for 10 hours unless otherwise noted. After 10 hours the samples were removed and immediately immersed in an ice bath to stop the hydrolysis. Next the samples were filtered through coarse sintered glass filters and the residual pulp was dried for 36 hours for weight loss determination.

Crystallinity Measurements

0.150 g of pulp was compressed into an aluminum sample holder under a pressure of 68.9 bar to form a disc of 15.9 mm in diameter. Both applied pressure and sample weight were chosen to be in a range where small fluctuations of these parameters have no influence on the peaks' intensity.[12]

Diffraction tracings were obtained using a Siemens 90-degree X-ray Diffractometer. A copper target was used for the X-ray beam generation with a characteristic wavelength = 1.54184 A (only Ka primary beam). The X-ray generator was operated at a constant voltage of 45 Kv and a current intensity of 20 mA was applied in the cathode circuit of the X-ray tube. The sample was scanned under the following conditions:

Scanning Range 2Θ:	10 to 30 degrees
Scanning Step =	0.1 degree
Exposure Time =	120 sec/ 0.1 degree

The Crystallinity Index (CI) which expresses the relative degree of crystallinity was calculated according to Segal, *et al.*[12]

Replication of Experiments

All experiments reported were replicated two or more times with the exception of the Kinetic Study and the conversions in the Crystallinity Indices Study.

RESULTS

Residual n-propylamine

Anhydrous n-propylamine was used to swell "buffered solvent" delignified pulp. The n-propylamine was removed from the pulp by vacuum evaporation, however, some residual n-propylamine remained in the pulp. The swollen pulp contained approximately 11 percent by weight residual n-propylamine. The n-propylamine in the pulp was neutralized by the addition of 1N acetic acid before enzymatic hydrolysis.

Enzymatic hydrolysis of the control pulp and the n-propylamine pretreated pulp resulted in a weight loss of 38.0±0.3 and 52.9±0.8% respectively. This 39 percent increase in enzymatic conversion for the pretreated pulp as compared to the control pulp was obtained in the presence of residual neutralized n-propylamine. However, amine containing compounds are known to inhibit a number of enzymes.

Therefore, a series of experiments were conducted to determine the impact of residual n-propylamine on the enzymatic hydrolysis of cellulose. In these experiments, neutralized n-propylamine was added to non-swollen buffered solvent pulp immediately before the addition of the enzyme solution. As can be seen in Figure 1, the residual neutralized n-propylamine had a significant inhibitory effect on enzymatic hydrolysis and must be extracted to maximize hydrolysis.

The type of solvent used for the extraction affects both the n-propylamine content of the solid residue and the swollen nature of the residue after drying. Residues treated with polar solvents such as water would be expected to have the lowest residual amine concentration. However water wetted, swollen cellulose is known to revert to crystalline CELLULOSE I upon drying.[13] Swollen cellulose wetted with less polar solvents such as acetone can retain their swollen nature upon drying.

A number of solvent extraction procedures were investigated to remove the n-propylamine from the pulp. The results of these experiments are shown in Table 1. The conversion increases as the propylamine content decreases. As expected the water extracted, never dried, material had the lowest n-propylamine content and the highest conversion.

Kinetic Study

A kinetic study was conducted using two different substrates: a control buffered solvent pulp and "propylamine pretreated-acetone extracted" buffered solvent pulp.

The results are shown in Table 2. For both the control and the swollen substrates, the rate of hydrolysis decreases gradually with time. This decrease is attributed primarily to the change in substrate accessibility over time. The easily accessible hemicellulose and amorphous cellulose are degraded rapidly, leaving more crystalline material in the reacting mixture.

The data in Table 2 can be modelled using "lumped" kinetic parameters as shown in Eq 1.[2] The overall inhibition constant, k, includes the effect of crystallinity hindrance, possible enzyme deactivation and product inhibition.

$$x = \frac{1}{k}\ell n(1 + \frac{V_o kt}{S_o}) \qquad (1)$$

where:

x	=	conversion (dimensionless)
k	=	overall inhibition constant (dimensionless)
V_o	=	initial hydrolysis rate (mM/hr)
S_o	=	initial concentration of cellulose (mM)
t	=	time of hydrolysis (hr)

The initial hydrolysis rate, V_o, was determined by plotting the hydrolysis rate data versus time and extrapolating to time 0. The overall inhibition constant, k, was determine by non-linear regression. The values obtained for both constants for pretreated and control pulp as given in Table 3. Equation 1 with the constants given in Table 3 has an excellent correlation with the experimental data shown in Figure 2.

X-ray Diffraction

X-ray diffraction tracings were obtained for different conditions of substrate pretreatment and for different extracting solvents. Also X-ray diffraction measurements were made for the residues of hydrolysis in order to determine the crystallinity of the remaining solids.

In Figure 3A, the diffraction tracing of milled poplar wood (40 mesh, raw material) with 7% moisture shows a broad and noisy background and non-intensive peaks for the planes $101,10\bar{1}$ and 002. A tracing of this type is expected considering the high lignin percentage (24%) and the subsequent low crystalline cellulose concentration of the sample. No crystallinity measurements were attempted for this sample.

The diffraction tracing of delignified buffered solvent pulp is shown in Figure 3B. Here the characteristic diffraction intensities from the $101,10\bar{1}$ and 002 planes are strong and relatively sharp. This tracing is characteristic of native CELLULOSE I. Diffraction tracings of n-propylamine treated pulp are significantly different. As shown in Figure 3C (pulp treated with n-propylamine and vacuum dried), the characteristic $101,10\bar{1}$ broad peak almost disappeared and the 002 sharp peak shifted from $2\Theta = 22.5°$ to $2\Theta = 21.7°$ and had a much lower intensity. This tracing is characteristic of CELLULOSE III.[11]

In Figure 3D, the influence of acetone extraction-air drying on the propylamine pretreated substrate is shown. A comparison between Figure 3C and Figure 3D indicates the CELLULOSE III structure was maintained. Only a faint broadening of the area under the 002 peak towards the higher scanning angle and a rather elevated area around $2\Theta = 15°$ could be detected. This could mean a small portion of CELLULOSE III was reconverted into CELLULOSE I. No quantification of this reconversion was possible. Thus, when the swelling agent is removed by a solvent such as acetone, the air-dried swollen cellulose retains to a significant degree its distended and amorphous condition.

When the residue containing n-propylamine is extracted using water and then air dried, even if washed with acetone the crystalline cellulose in the dried residue is CELLULOSE I (see Figures 3E and 3F). The characteristic diffraction peaks are still relatively decreased in comparison with Figure 3B. In the case where acetone was used to reextract the water before air drying the substrate, a relative retention of the swollen state is observed. Loeb and Segal reported similar results for an analogous system.[13]

Finally, Figure 4 presents the diffractogram of the residue of hydrolyzed n-propylamine pretreated pulp. Crystallinity has increased after hydrolysis. It is important to remember that the lignin concentration has substantially increased compared to the non-hydrolyzed sample. This means the carbohydrate fraction in the residue is largely crystalline.

Crystallinity Indices for hydrolyzed and non-hydrolyzed material were determined and are shown in Table 4. The crystallinity index represents a relative and not an absolute measurement of crystallinity. The n-propylamine swollen pulp had a significantly reduced CI as compared to the control (untreated) pulp. As expected, the CI increased substantially for all the substrates after hydrolysis.

Using the CI and the lignin content for two of the cases shown in Table 4, the relative composition of pulp and hydrolyzed residue was calculated. As shown in Table 5, the carbohydrate remaining in the n-propylamine pretreated residue after hydrolysis was highly crystalline.

CONCLUSIONS

Pretreatment of delignified buffered solvent pulp with n-propylamine swells the CELLULOSE I structure present in native cellulose and decreases the crystallinity of the pulp. The crystalline cellulose which remains after swelling is in the form of CELLULOSE III. If the pretreated pulp is contacted with water and dried, the CELLULOSE III crystals revert to CELLULOSE I.

Pretreatment of delignified buffered solvent pulp with n-propylamine can increase the enzymatic conversion of the pulp by approximately 60 percent. Cellulases are strongly inhibited by n-propylamine. Extraction of the residual n-propylamine from the pretreated pulp while maintaining the swollen state is required for maximum conversion.

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REFERENCES

1. Ogiwara, Y., and K. Arai, *Text. Res. Journal*, 38, p. 885 (1968).
2. Ohmine, K, H. Oshima, and Y. Harano, *Biotechnol. and Bioeng.*, 25, p. 2041 (1983).
3. Faass, G. S., R. S. Roberts, and J. D. Muzzy, *Holzforschung*, 43:4, p. 245 (1989).
4. Betrabet, S. M., and M. L. Rollins, *Text. Res. Journal*, 40, p. 917 (1970).
5. Modi, J. R., S. S. Trivedi, and P. C. Mehta, *J. Appl. Poly. Science*, 7, p. 15 (1963).
6. Pandey, S. N., and R. L. N. Iyengar, *Text. Res. Journal*, 39:24, p. 15 (1969).
7. Pandey, S. N., and R. L. N. Iyengar, *Text. Res. Journal*, 39, p. 24 (1969).
8. Holtzapple, M. T., Doctoral Thesis, University of Pennsylvania, Philadelphia, PA (1981).
9. Barry, A. J., F. C. Peterson, and A. J. King, *J. of Am. Chem. Soc.*, 58, p. 333 (1936).
10. Marrinan, H. J., and J. Mann, *J. Polym. Science*, 21, p. 301 (1956).
11. Patil, N. B., P. K. Chidambareswaran, H. T. Lokhande, and S. R. Shukla, *J. Polym. Science*, 15, p. 97 (1977).
12. Segal, L., J. J. Creely, A. E. Martin, Jr., and C. M. Conrad, *Text. Res. Journal*, 29, p. 786 (1959).
13. Loeb, L., and L. Segal, *J. Polym. Science*, 15, p. 343 (1955).

Figure Legends

Figure 1: Effect of Added Neutralized Propylamine on Enzymatic Hydrolysis of Buffered Solvent Pulp.

Figure 2: Enzymatic Hydrolysis Experimental and Calculated Data.
□ = Control Buffered Solvent Pulp Experimental Data

△ = Propylamine Treated, Acetone Extracted, Buffered Solvent Pulp Experimental Data

• = Control Buffered Solvent Pulp Calculated Using Model

∇ = Propylamine Treated, Acetone Extracted, Buffered Solvent Pulp Calculated Using Model

Figure 3: X-Ray Diffactograms of:
A: Milled Poplar Wood–40 Mesh.

B: Buffered Solvent Pulp.

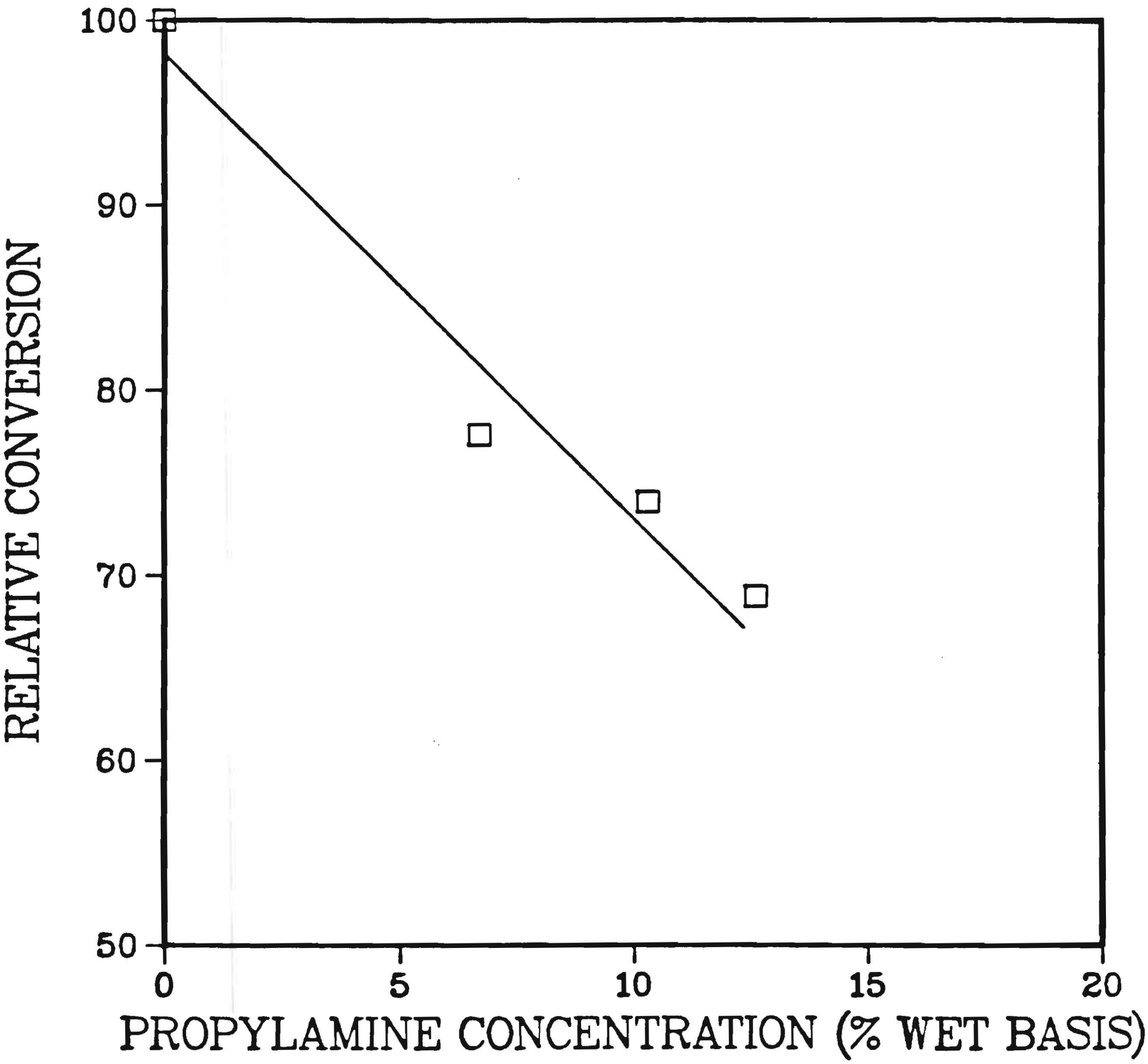
C: n-Propylamine Swollen, Vacuum Dried, Buffered Solvent Pulp.

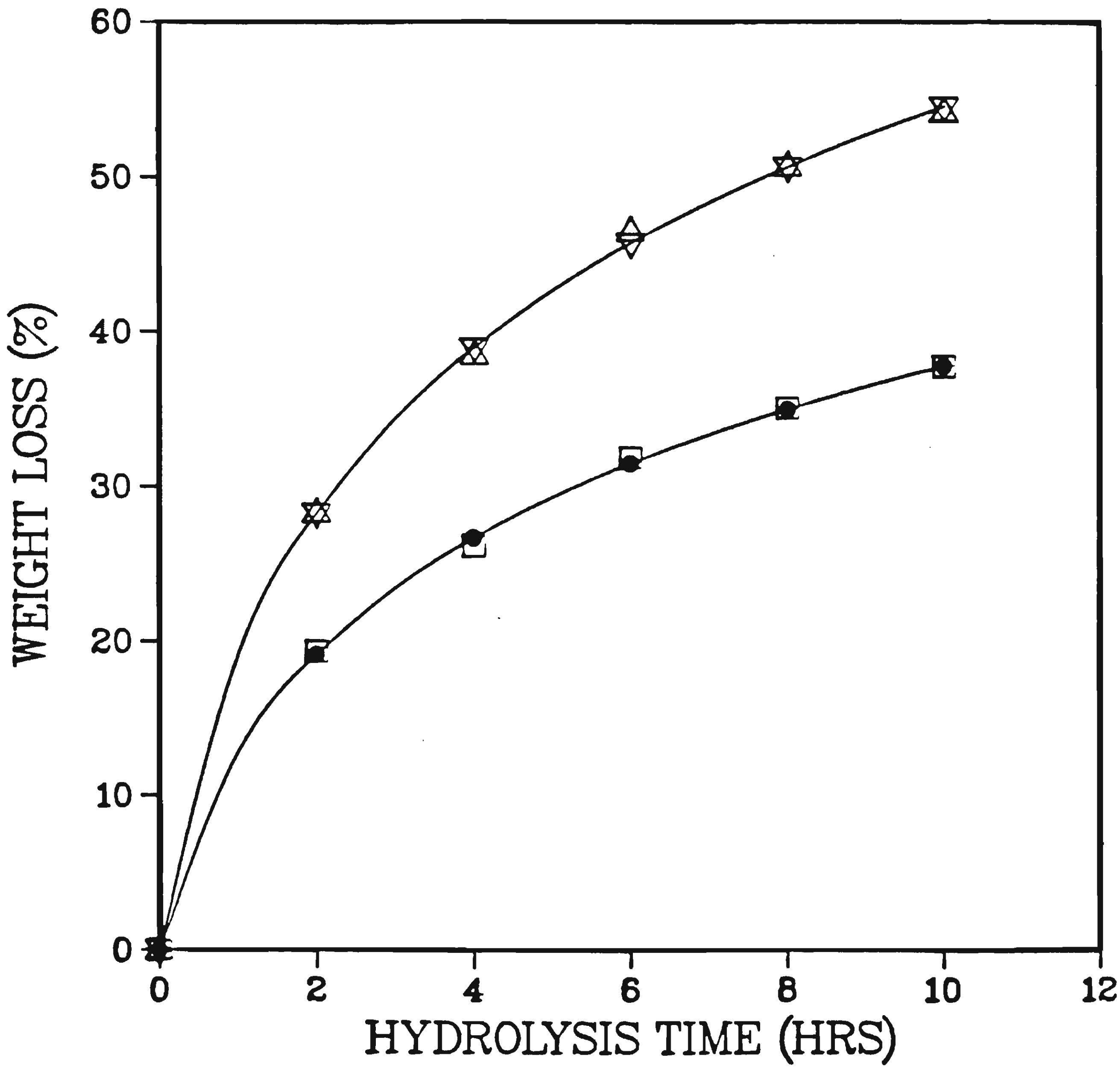
D: n-Propylamine Swollen - Acetone Extracted, Buffered Solvent Pulp.

E: n-Propylamine Swollen, Treated - Water Extracted - Air Dried, Buffered Solvent Pulp.

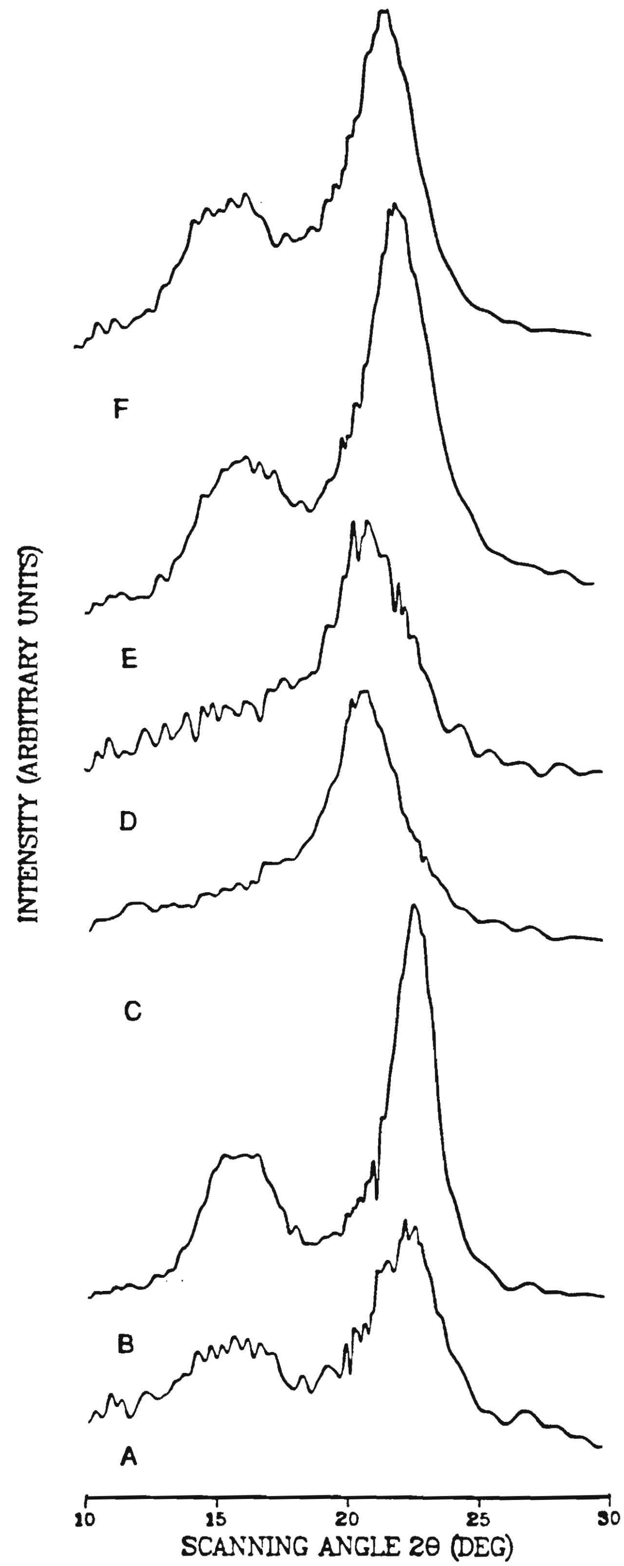
F: n-Proplyamine Swollen - Water Extracted - Acetone Washed and Air Dried, Buffered Solvent Pulp.

Figure 4: X–Ray Diffractogram of Standard Hydrolysis Residue (Substrate: n-Proplyamine Swollen, Water Extracted, Buffered Solvent Pulp.)


100
90
80
70
60
50
0
5
10
15
20
RELATIVE CONVERSION
PROPYLAMINE CONCENTRATION (% WET BASIS)



60
50
40
30
20
10
0
WEIGHT LOSS (%)
0
2
4
6
8
10
12
HYDROLYSIS TIME (HRS)



INTENSITY (ARBITRARY UNITS)
F
E
D
C
B
A
10
15
20
25
30
SCANNING ANGLE 2θ (DEG)

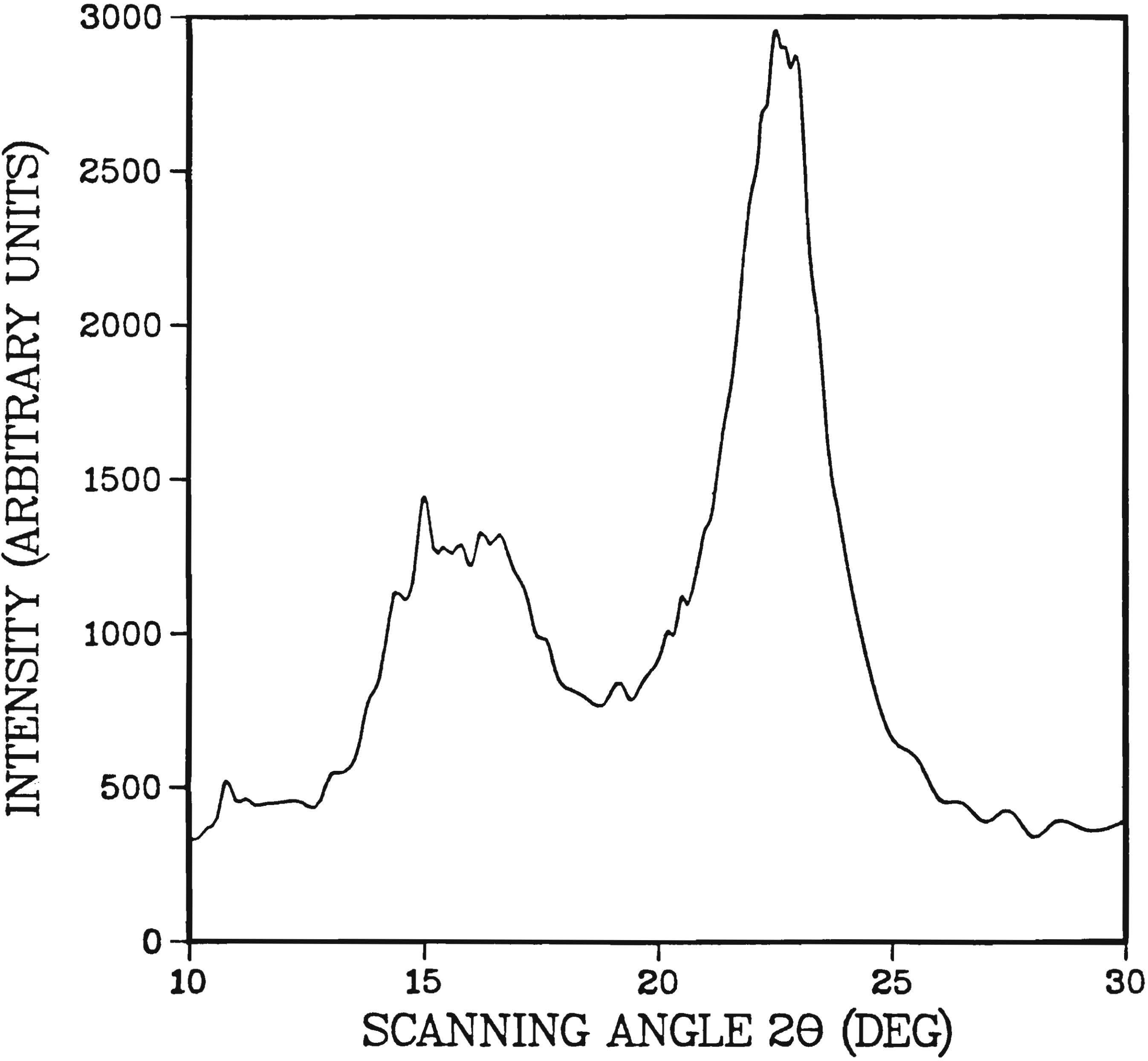

3000
2500
2000
1500
1000
500
0
INTENSITY (ARBITRARY UNITS)
10
15
20
25
30
SCANNING ANGLE 2θ (DEG)

TABLE 1

The Influence of Solvent Extraction
on the Enzymatic Hydrolysis of
n-Propylamine Pretreated Pulp

Substrate Conversion	Moisture (% wt)	Propylamine(*) content (% wt)	Conversion (% wt loss)
Untreated control	9.4 ± 0.3	—	38.0±0.3
Propylamine treated	13.5 ± 0.4	11.1 ± 1.9	52.9±0.8
Propylamine treated + acetone extracted air dried	6.5 ± 0.2	2.2 ± 0.4	55.7±0.9
Propylamine treated + ethanol extracted air dried	7.0 ± 0.3	0.7 ± 0.2	59.1±1.2
Propylamine treated + methanol extracted air dried	6.1 ± 0.2	0.5 ± 0.2	58.3±0.5
Propylamine treated + water extracted + never dried	49.7 ± 1.3	0.0	60.7±1.0

(*) Propylamine content was calculated by titration with 0.1 N HCl acid, using phenol-phtalein as indicator.

TABLE 2

Hydrolysis Rate Data

Hydrolysis Time (Hrs.)	Control Pulp % wt. loss Total	Control Pulp % wt. loss Δ^1	n-Propylamine Pretreated Pulp* % wt. loss Total	n-Propylamine Pretreated Pulp* % wt. loss Δ^1	$\frac{\Delta^1 \text{ Propyl Pretreated}}{\Delta^1 \text{ Control}}$
2	19.34	19.34	28.25	28.25	1.46
4	26.14	6.80	38.45	10.20	1.50
6	31.81	5.67	46.43	7.98	1.41
8	35.03	3.22	50.65	4.22	1.31
10	37.71	2.68	54.14	3.49	1.30

$^1\Delta$ = Total % wt loss at $time_{i+1}$ - Total % wt loss $time_i$

Additional Information:

	Moisture (%)	Propylamine Content (%)
Untreated control	10.10	—
Propylamine pretreated-acetone extracted	8.85	2.9

TABLE 3

Enzymatic Hydrolysis
Rate Equation Constants

Substrate	K(dimensionless)	$V_o(mM/\mathrm{hr})$
Pulp 220/20 untreated control	7.15	31.25
Pulp 220/20 propylamine treated, acetone extracted	5.13	48.66

TABLE 4

Crystallinity Indices (CI) of Swollen
Hydrolyzed and Non-hydrolyzed Pulp

Substrate	Non-hydrolyzed CI (%)	Hydrolyzed CI (%)	Conversion (% wt loss)
Untreated control	78.2±0.7	81.4±0.2	38.2
Propylamine treated	44.1±1.2	N/A	N/A
Propylamine treated water extracted + air dried	63.8±0.4	75.6±0.8	55.3
Propylamine treated water extracted acetone washed air dried	61.7±0.8	71.5±0.4	58.0
Propylamine treated water extracted + never dried	N/A	71.3±1.3	61.4

N/A not available

TABLE 5

Characteristics of Pulp Before and After Hydrolysis

	Control Pulp				n-Propylamine Pretreated Pulp*			
	Crystalline Cellulose	Amorphous Carbohydrate	Lignin	Total	Crystalline Cellulose	Amorphous Carbohydrate	Lignin	Total
Non-hydrolyzed Pulp	78.2g 78.2%	16.9g 16.9%	5.0g 5.0%	100g 100%	61.7g 61.7%	33.3g 33.3%	5.0g 5.0%	100g 100%
Residue after hydrolysis	50.5g 81.4%	6.5g 10.5%	5.0g 8.1%	62g 100%	30.4g 71.5%	6.6g 15.7%	5.0g 11.9%	42g 100%

*water extracted, acetone washed, air dried pulp